SUPPLEMENTARY MATERIAL

Circulating microbiome methodology

The DNA was extracted from whole blood (200 µL) using a DNA isolation kit (NucleoSpin blood kit, Macherey-Nagel, Duren, Germany) after three mechanical lysis cycles for 30 seconds at 30 Hz in a bead beater (TissueLyser,Qiagen, Hilden, Germany) with 0.1 mm glass beads (MoBio Laboratories, Inc., Carlsbad, CA) to increase the yield of bacterial DNA. The quality and quantity of extracted DNA were monitored by gel electrophoresis (1% agarose in TBE 0.5x) and ultraviolet spectrophotometer (Nano-Drop 2000, Thermo Scientific, Wilmington, DE).

Following DNA extraction from whole blood, 16S qPCR DNA was quantified. The relative proportions of 16S rDNA bacterial profile were determined using next generation high throughput sequencing of variable regions (V3-V4) of the 16S rDNA bacterial gene. The organisms were classified from metagenomic samples by amplifying specific regions in the 16S ribosomal RNA genes exclusive to bacteria. The main output was a classification of reads at several taxonomic levels: phylum, class, order, family and genus. The targeted metagenomics workflow included the following steps:

a) *Library construction and sequencing*

PCR amplification was performed using 16S universal primers targeting the V3-V4 region of the bacterial 16S ribosomal gene. The joint pair length was set to encompass 476 base pairs amplicon thanks to 2 x 300 paired-end MiSeq kit V3. For each sample, a sequencing library was generated by addition of sequencing adapters. The detection of the sequencing fragments was performed using MiSeq Illumina® technology.

b) Bioinformatics pipeline

After demultiplexing of the bar coded Illumina paired reads, single read sequences were cleaned and paired for each sample independently into longer fragments. After quality-filtering and alignment against a 16S reference database (MOTHUR v1.34.4 with RDP training set v9), clustering into operational taxonomic units (OTU) with a 97% identity threshold, and a taxonomic assignment were performed in order to determine community profiles. The targeted metagenomic sequences from microbiota were used for taxonomic classification and to derive microbiota composition and diversity.

c) 16S qPCR with extracted samples

The DNA extracted for sequencing was evaluated by qPCR using the same base primers for sequencing the V3-V4 16S rDNA region. The total 16S rDNA present in the samples was measured by qPCR in triplicate and normalized using a plasmid-based standard scale. The construction of a standard curves allowed for a proper quantification of 16S rDNA gene copy in the sample, but also enabled the determination of the efficiency, linear dynamic range, and reproducibility of the qPCR assay. In these experiments, the efficiency calculated from the standard curve was required to be between 80-120%, and the R2 of the standard curve greater than 0.980.

Melting curve analysis is the assessment of heat induced dissociation-characteristics for double-stranded DNA. Melting curve analysis helped ensure the specificity of the target PCR amplicons, including identification of the presence of non-specific products and primer-dimers. This property is valuable because the presence of secondary non-specific products and primer-dimers can affect the accuracy of the qPCR

assay. The specificity of all qPCR products was assessed by systematic analysis of the post-PCR dissociation curve performed between 60°C to 95°C.

Circulating LPS, LBP and sCD14:

Endotoxin or lipopolysaccharide (LPS) concentrations were assessed using the Limulus Amebocyte Lysate (LAL) kinetic chromogenic methodology (Charles River, Lyon, France). Lipopolysaccharide (LPS) Binding Protein (LBP) plays a central role in the response to LPS and LBP concentrations were assessed by the human LBP ELISA (Hycult Biotech, The Netherlands). Soluble CD14 concentrations were assessed by human sCD14 ELISA (Hycult Biotech, The Netherlands). All experiments were performed using the same lots of reagents for the entire study.

SUPPLEMENTARY FIGURE:

Supplementary Fig. 1

Plasma endotoxin or lipopolysaccharide (LPS) levels among the study groups

Supplementary Fig. 2

Gender and circulating microbiome: Cladograms representing significant enrichment of circulating microbiome based on gender within no alcohol control (NAC) group (Fig. 2A) and all alcohol drinking groups (HDC, MAH and SAH, all combined) in Fig. 2B.

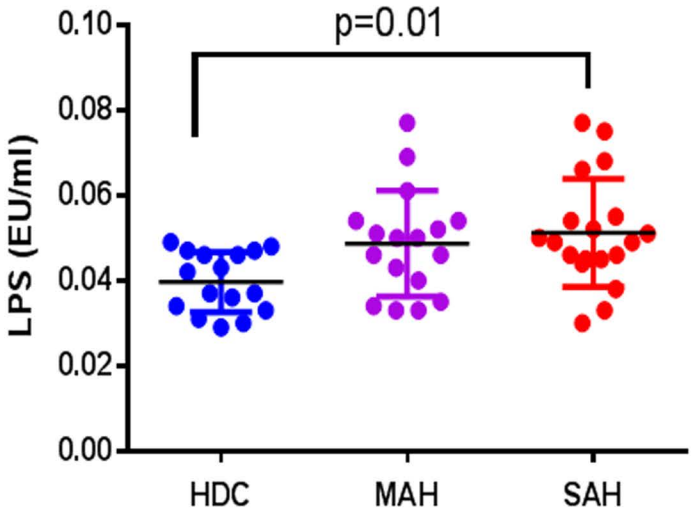
HDC: Heavy Drinking Controls; MAH: Moderate Alcoholic Hepatitis; and SAH: Severe Alcoholic Hepatitis

Supplementary Fig. 3

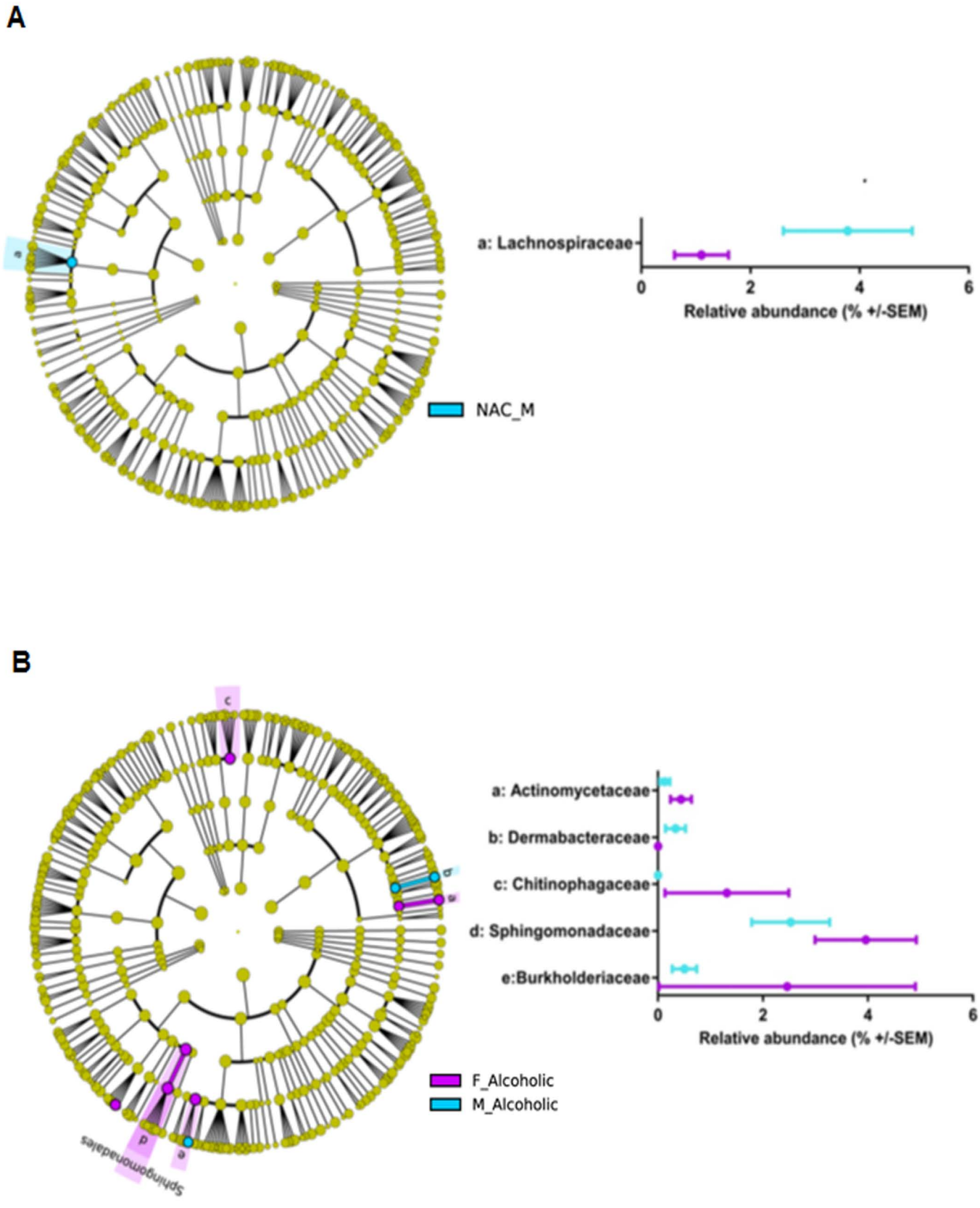
Circulating microbiome in no antibiotic exposure subjects: Cladograms in Fig. 3A and 3B demonstrate significant enrichment of specific circulating microbiota in subjects with no antibiotic exposure. In Fig. 3A, no alcohol control (NAC) vs. heavy drinking controls (HDC) vs. Alcoholic Hepatitis (AH) groups were compared. The NAC group had high relative abundance of Bacteroidetes. Also, subjects without antibiotics use in HDC and AH groups were enriched with families Peptostreptococcaceae, Dermabacteraceae and Streptococcaceae. Next, we compared the microbiota composition read outs between NAC [n= 20] and all alcoholics with no antibiotic use [n=25] (Fig. 3B). The NAC group was enriched in Bacteroidetes and alcoholics with no antibiotic use were enriched in Fusobacteria. Moreover, alcoholics without antibiotics were enriched with Peptostreptococcaceae, Leptotrichiaceae, Bradyrhizobiaceae, and Clostridiales Incertae

Sedis XI. Observations in both Fig. 3A and 3B represent similar findings as noted in the original cohort that included subjects with and without antibiotic use.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3